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Enolases: Limitations for Implementation in Clinical Practice (Critical Review)

Arkady M. Golubev*

V. A. Negovsky Research Institute of General Reanimatology,
Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology,
25 Petrovka Str., Bldg. 2, 107031 Moscow, Russia

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***Correspondence to:** Arkady M. Golubev, arkadygolubev@mail.ru

Summary

Enolases (ENOs) are involved in glycolysis, which is critical for providing energy to cells under hypoxic conditions. ENOs are attracting the attention of researchers as a potential diagnostic marker for critical conditions.

The aim of this review is to analyze the reasons limiting the clinical use of ENOs for diagnostic and prognostic purposes in critical conditions.

We selected and analyzed 87 publications in which ENOs assessment was mainly performed in patients with critical illness. Criteria for selecting relevant publications from PubMed and Elibrary were based on a presence of authors' recommendations or current guidelines on clinical use of ENOs for diagnostic or prognostic purposes.

Specific properties of human ENO isoenzymes were reviewed, clinical aspects and recommendations for their clinical use, as well as methodological and procedural errors in ENO testing were considered.

The following controversial issues were identified: the measured level of ENOs does not characterize the true enzymatic activity of their numerous molecular isoforms; identification of specific ENO isoforms using antibodies to structural subunits does not allow assessment of the true content and enzymatic activity of potentially circulating isoenzymes (e.g., gamma-gamma and alpha-gamma ENOs); the concept of cell specificity ascribed to heterodimers (in particular, gamma-alpha enolase is considered to be neuron-specific) is not supported by the results of the available studies, since this heterodimeric form of ENO is present in a variety of human tissues; some procedural issues are not taken into account (e. g., latent hemolysis, lack of standardized assessment methods, etc.).

Conclusion. The use of advanced diagnostic platforms based on the assessment of the content and enzymatic activity of each ENO isoform should provide valuable information on their specific role in the pathogenesis of diseases in the context of personalized medicine and will enable the evaluation of their diagnostic and prognostic significance, as well as the effectiveness of therapeutic interventions.

Keywords: enolases; isoenzymes; multiple molecular forms; critical conditions; clinical guidelines

Conflict of interest. The authors declare no conflict of interest.

Introduction

Various diseases and lesions leading to critical illness are characterized by severe metabolic derangements, making molecular biomarker research important for disease diagnosis, management, and prognosis [1–7].

Enolase is a promising molecular biomarker. The discovery of glycolysis, one of the primary mechanisms of glucose oxidation (the Embden–Meyerhof–Parnas pathway), prompted active research into enolases. This pathway is based on the phosphorylation of glucose, resulting in the production of pyruvate. In 1940, researchers discovered the glycolytic reactions that produce ATP and NADH, which provide energy for cellular metabolism.

Meyerhoff and Lohmann discovered enolase in the early 1930s while studying glycolysis [8]. Phosphopyruvate hydratase, also known as 2-phospho-D-glycerate hydrolase (EC 4.2.1.11) and commonly referred to as enolase, is the primary catabolic enzyme that converts 2-phosphoglycerate to phosphoenolpyruvate in the glycolytic pathway for ATP production [9].

Under normal oxygen conditions, glycolytic enzymes are widely distributed throughout the cytoplasm of cells. Recent research has revealed the compartmentalization of glycolytic enzymes in response to hypoxic stress. A higher concentration of glycolytic enzymes increases the rate of glucose utilization, which boosts energy production. Disruption of compartmentalization can impair metabolism (even in neurons). Hypoxia inhibits the tricarboxylic acid cycle, making glycolysis the primary pathway for converting glucose into usable energy. However, the mechanisms that compensate for the loss of energy production caused by the inactivation of the tricarboxylic acid cycle are still poorly understood [10].

The importance of studying glycolysis for diagnostic purposes in medical practice is undeniable. In this context, enolase has attracted attention due to its potential use as a diagnostic marker in critical illness.

The aim of the review is to analyze the factors limiting clinical use of enolase for diagnostic and prognostic purposes in critical illness.

We analyzed more than 500 literature sources on enolase. 87 publications from PubMed and eLibrary databases were selected based on the authors' suggestions for the use of enolase research results for diagnostic purposes, as well as official recommendations for the use of enolases in clinical practice.

The critical review discusses the molecular forms of enolases, clinical aspects and recommendations for their clinical use, and methodological errors in the study of enolases.

Limitations of the studies included in the review: we primarily used publications on the study of enolases in critical illness.

Molecular Variants of Enolases

Enolases are highly expressed and conserved proteins with identical amino acid sequences in all organisms, from archaea to mammals [11]. According to Seki S. M. and Gaultier A., enolase activates glycolysis, which plays an important role in metabolism during inflammation and hypoxia [12].

The enolase family includes multiple molecular forms of enzymes (MMFE), a class of protein metalloenzymes with identical catalytic functions but different structures, physical and chemical properties. The diversity of MMFEs serves an important biological function. When environmental conditions change, the cell's MMFE spectrum shifts, allowing the organism to adapt more effectively. Changes in MMFEs, such as their number, activity of each form, and stability, are one of the mechanisms that control metabolic processes.

Among the MMFEs of enolases, there are genetically determined forms known as isoenzymes, which differ from each other in the primary structure of the protein. In addition, there are forms that result from epigenetic changes.

All molecular forms of enolase are found primarily in the cytosol. The short variable regions in the structure of enolases serve as contacts with cytoskeletal elements and ensure their precise localization.

Enolase isoenzymes consist of three subunits (a, β, and γ), which combine to form homo- or heterodimers.

Homodimers are composed of 2 identical subunits, including

- enolase 1 (ENO 1), or α-enolase, consisting of two α-subunits ($\alpha\alpha$)
- enolase 2 (ENO 2), or γ-enolase (also called neuron-specific enolase, NSE), consisting of two γ subunits (γγ)
- enolase 3 (ENO 3), or β-enolase, consisting of two β-subunits (ββ).

Heterodimers include $\alpha\gamma$ (NSE isoenzyme) and βγ isoforms.

ENO-S, another enolase isoenzyme, has been found in human, ram, and mouse spermatozoa. This isoenzyme is unique to spermatozoa and differs from the somatic enolases ENO 1, ENO 2, and ENO 3 in its electrophoretic mobility, high thermostability, and ability to undergo structural changes at high temperatures. It was named enolase 4 (ENO 4) [13].

A study of ENO-S expression during sperm differentiation suggested that this isoenzyme is synthesized relatively late in the haploid genome [14].

All possible dimers except βγ were detected in vivo. The molecular masses of the enolases range from 82 to 100 kDa [15].

Different genes encode the α, β and γ subunits: ENO 1 for α-enolase, ENO 2 for γ-enolase, and ENO 3 for β-enolase. Their expression changes during development, metabolic or general pathological processes [16].

Bivalent metal ions, particularly Mg^{2+}, are required for the structural stability and catalytic activity of enolase. The substrate 2-phosphoglycerate binds to the active site of enolase, which uses metallic magnesium ions as cofactors. Zn^{2+} ions have the same effect. Fluoride inhibits the enolase enzyme [17]. Molecular forms of enolases perform both enzymatic and non-enzymatic functions.

Biologic Role and Clinical Aspects of Enolases

The optimal use of a biomarker in medical practice requires high specificity and sensitivity, as well as a rapid and inexpensive method of measurement [18].

Alpha-enolase. Alpha-enolase is involved in pyruvate synthesis, acts as a plasminogen receptor, and facilitates plasmin activation and extracellular matrix degradation. Alpha-enolase is found on the surface of many cell types and contributes to tumor invasion.

α-Enolase is expressed on the cell surface of differentiating myocytes, and inhibitors of α-enolase-plasminogen binding prevent skeletal muscle regeneration [19].

The association of α-enolase with the mitochondrial membrane promotes membrane stability, while its sequestration on the cell surface is critical for plasmin-mediated pericellular proteolysis [20].

Starting from the embryonic period, α-enolase continues to be expressed in most adult tissues. Specific isoforms of enolase develop during ontogeny

in two cell types with high energy requirements: αγ- and γ-enolase are detected in neurons, αβ- and β-enolase in transverse striated muscle cells [21].

The embryonic brain has a high level of α-enolase, which decreases as neurons mature [22]. During brain differentiation, α-enolase is replaced by γ-enolase in neurons, which is a late event in nervous system development and may be a marker of neuronal maturation [23].

Overexpression of α-enolase and its post-translational modifications (acetylation, methylation, and phosphorylation) may have diagnostic and prognostic significance in many cancers. The ability of α-enolase to induce a potent specific humoral and cellular immune response makes this protein a promising target for tumor therapy [24, 25].

Overexpression of human α-enolase has been reported in a wide range of cancers and is closely associated with poor prognosis, making it a potential therapeutic target and biomarker [26].

α-Enolase is overexpressed in cutaneous melanoma cells. Overexpression of α-enolase led to increased tumor cell invasion, migration and proliferation, as well as higher pyruvate and lactate levels. Melanoma cells with disabled α-enolase showed the opposite effects. Thus, α-enolase is a potential therapeutic target in cutaneous melanoma [27].

Alpha-enolase stimulates the growth of pancreatic cancer cells. Knockout of α-enolase expression inhibited tumor cell proliferation and colony forming ability. One study found that α-enolase is an oncogenic biomarker and a potential target for immunotherapy in pancreatic cancer [28].

Another study investigated how α-enolase affects the proliferation, invasion and apoptosis of human breast cancer cells. The study suggested that α-enolase may be a therapeutic target in breast cancer [29]. Suppression of its expression reduces the proliferative activity, invasion ability, and apoptosis rate of breast cancer cells.

α-Enolase interacts with β-amyloid (Aβ) and inhibits fibril formation [30]. Proteolytic degradation of the Aβ peptide effectively destroys Aβ fibrils and reduces their cytotoxic effects. Infusing α-enolase into the brains of APP23 mice reduced Aβ deposition and cognitive impairment.

Enzymatically inactivated α-enolase was unable to inhibit the formation and destruction of Aβ fibrils. α-enolase's proteolytic activity may contribute to its cytoprotective effects and clearance of Aβ from the brain, making it a potential therapeutic target for cerebral amyloid angiopathy [30].

Several non-metabolic functions of α-enolase impact viral replication in infected cells, leading to research on its potential use as a target for treating viral diseases [31].

Gamma-enolase. Researchers and clinicians are interested in gamma-enolase (NSE), also known as αγ isoenzyme, as it is thought to be a neuron-specific marker [18].

In neurons and neuroendocrine cells, γ-enolase is associated with the plasma membrane. NSE is involved in axonal transport and its expression level varies according to the energy needs of the cell. When axons are damaged, NSE levels increase. NSE immunohistochemistry selectively labels damaged axons in the corpus callosum in diffuse axonal injury, whereas NSE is not detected in intact axons [32].

Serum NSE concentrations are higher in patients with ischemic stroke than in controls and correlate with infarct size and neurological deficits [33, 34]. Patients with a second peak of elevated serum NSE in the late phase of ischemic stroke (20%) were more likely to experience hemorrhagic transformation [35].

An NSE level of less than 2 ng/mL in the acute phase of stroke predicts a good functional outcome 12–14 days after stroke onset. NSE levels above 2.6 ng/mL are associated with a high risk of death [36]. In both young and elderly ischemic stroke patients who improved, NSE levels were either stable or decreased at the time of hospital discharge. At the same time, NSE levels increased in patients who had a poor outcome [37].

Serum NSE level is an indicator of neuronal damage and helps to predict disability and clinical outcome in patients with hypertension and ischemic stroke [38]. It has been proposed to use serum NSE levels as a biochemical marker of damage in cerebral ischemia-reperfusion after carotid endarterectomy [39].

The detection of NSE in peripheral blood may provide valuable and timely diagnostic information about stroke, especially when the time of stroke onset cannot be determined [40]. Given the correlation between serum NSE levels and cerebral infarct size, NSE may be a predictor of severe clinical manifestations of acute ischemic stroke [41]. High baseline NSE levels are associated with poor outcomes of ischemic stroke within 1 year in patients with hypertension [42].

Patients with out-of-hospital cardiac arrest who underwent body temperature management with adverse neurological outcomes were reported to have higher serum NSE concentrations with severe blood-brain barrier abnormalities than without [43].

The combination of NSE measurement and neuroimaging improves prediction of outcome after cardiac arrest with targeted body temperature management [44]. Cardiac arrest was reported in 171 of 475 patients (36%), with good neurological outcomes at 6 months for low NSE levels and poor outcomes at 6 months for high NSE levels [45].

High levels of NSE in cerebrospinal fluid were found to be a predictor of poor neurological outcome in survivors of out-of-hospital cardiac arrest [46]. In non-survivors of out-of-hospital cardiac

arrest, serum NSE levels increased during the first 72 hours, and in survivors, NSE levels decreased after 48 hours [47].

Elevated serum NSE levels have been observed in tuberculosis, chronic obstructive pulmonary disease, alveolar proteinosis, and acute respiratory distress syndrome [15]. Elevated serum NSE concentrations have been reported in patients with silicosis, which is important for diagnosis and assessment of disease severity [48].

Patients with dyspnea in SARS-CoV-2 infection have higher serum NSE levels than patients with milder disease and controls [49].

Elevated serum NSE levels have been reported in patients with small cell lung cancer [50]. NSE modulation has been shown to regulate cell proliferation, drug resistance and tumor growth [51].

NSE has been suggested as a candidate biomarker for gastric cancer prognosis [52].

However, there has been an increase in the number of publications showing results that do not support the clinical efficacy of enolase monitoring (primarily NSE).

Due to the difficulty in interpreting the results of NSE studies, a coefficient has been proposed as a method for quantitative assessment of changes in its level, eliminating the need to assess the absolute values of NSE. Coefficient values greater than 1.0 indicate an increase in NSE concentration, which could indicate progressive neuronal damage [53].

However, A. Huţanu et al. [54] questioned the use of NSE as a marker for ischemic stroke. In this study, there were no significant differences between serum NSE levels in ischemic stroke patients and controls, and a high NSE level was associated with a better outcome. In addition, NSE levels were not associated with functional outcome after three months.

A systematic review found no association between NSE levels and functional outcome or stroke severity [55]. NSE did not help discriminate between ischemic and hemorrhagic stroke [56].

There have been conflicting findings regarding the significance of NSE levels in the late phase of ischemic stroke after endovascular treatment. Blood samples were taken from 90 patients before endovascular treatment and at 2 hours, 24 hours, 48 hours, 72 hours and 3 months after treatment. Serum NSE levels remained constant throughout the study [57].

L. E. Pelinka et al. [58] found interesting results based on clinical and experimental data in a study aimed at answering the question whether NSE is an informative early marker of traumatic brain injury (TBI) and whether NSE affects ischemia/reperfusion injury of abdominal organs. It has been shown that serum NSE levels are elevated to the same extent in patients with and without polytrauma, but without TBI.

In rats, serum NSE concentrations increased more than threefold during hepatic and renal ischemia and more than two to three times after hepatic, renal, and intestinal reperfusion compared with laboratory controls. Thus, the hypothesis that NSE is an early indicator of TBI in multiple trauma was not supported [58].

Beta enolase. Beta-enolase is present in both skeletal and cardiac muscle [21, 59]. High levels of β-enolase subunits are characteristic of rapidly contracting fibers of adult muscle [60]. Beta-enolase is a marker of muscle differentiation in rhabdomyosarcoma [61].

Mutations of the *ENO3* gene, which produces the β-subunit of enolase, produce β-enolase with low stability [62]. Serum β-enolase levels are indicative of exercise-induced muscle damage in athletes [63].

Muscle β-enolase deficiency is a very rare inherited metabolic myopathy. In one study, two men, one Italian and one Turkish, whose parents were blood relatives, had several episodes of severe myalgia, cramps, generalized muscle pain, and dark urine. None of the other family members reported similar symptoms. Biochemical studies of muscle tissue showed a marked decrease in muscle β-enolase activity (20 and 10% residual activity, respectively). Molecular genetic analysis of the *ENO3* gene revealed two homozygous missense mutations [64].

Immunocytochemical analysis of transverse sections of adult mouse calf muscle allowed the expression of α- and β-subunits to be compared with the expression of myosin heavy chain isoforms. The expression of β-enolase in muscle cells is finely regulated in response to energy demands. The intensity of α-enolase expression appeared to be independent of fiber type. Confocal microscopy analysis showed that α-enolase was localized in the M-band. Most of the β-enolase was distributed throughout the sarcoplasm. Some β-enolase was localized in both the Z- and M-bands. The results of the study support the idea that isoenzymes differ in their ability to interact with other macromolecules and partition to different subcellular locations where they respond to specific functional needs [65].

ENO-S (sperm specific enolase). The ENO-S isoenzyme has been studied in spermatozoa at different stages of maturation. The electrophoresis method showed that in testicular spermatozoa ENO-S was present in 2 major bands, named S1 and S3. When ENO-S was analyzed in spermatozoa from the seminiferous tubules, bands S1, S3 and an additional band S2 were visualized, which had the same electrophoretic properties as ENO-S from ejaculated sperm.

None of the 3 ENO-S bands were detected in testicular extracts in which spermatozoa were not visualized by histological analysis. Thus, ENO-S

exists as different isoforms (electrophoretic variants) at different stages of sperm maturation. The passage through the seminiferous tubules seems to play an important role in the maturation process of ENO-S [66].

ENO-S and α-enolase. Total ENO-S and α-enolase levels of spermatozoa were measured in 30 normospermic fertile men and 20 patients with abnormospermic infertility. Total enolase level was significantly higher in total spermatozoa of patients with sperm abnormalities compared to normospermic patients. The α-enolase level in total sperm was significantly higher in abnormal than in normospermic men. The α-enolase concentration correlated positively with the percentage of immature spermatozoa with excess residual cytoplasm. The ENO-S level in total spermatozoa of normospermic patients was significantly higher than in abnormospermic patients. The studied enolase isoforms seem to reflect opposite aspects of sperm quality: α-enolase is associated with abnormal and ENO-S with normal sperm. As an additional parameter to distinguish normal from abnormal sperm, the ENO-S : α-enolase ratio was evaluated in both groups. This ratio is a marker of sperm quality and is a prognostic index of the potential of sperm to fertilize the oocyte [67].

Combining hetero- and homodimeric enolase forms. The majority of platelet and erythrocyte enolase is represented by the αγ heterodimer combined with the α-enolase homodimer [68, 69].

Two monoclonal antibodies against human and bovine γ-enolase were produced in isolated hybrid cell lines. They showed reactivity with γ and αγ isoforms of human and rat γ-enolase and with bovine γ-enolase. The antibodies did not cross-react with α- or β-subunit isoenzymes of human and rat enolase. Both antibodies partially inhibited γ- and αγ-enolase activity [70].

The distribution of 3 forms of rat enolase (α-, αγ- and γ-), including those specific to the nervous system (αγ- and γ-enolase), was determined using an enzyme-linked immunosorbent assay system. Brain and spinal cord contained more than 100 pmol/mg of α-, αγ- and γ-enolase. Organs such as lung, heart, spleen, liver and kidney had similarly high α-enolase concentrations, but αγ- and γ-enolase levels were less than 1% of their concentrations in the central nervous system. High concentrations of αγ- (greater than 10 pmol/mg) and γ-enolase (greater than 1.5 pmol/mg) were found in the rectum, bladder and uterus [71].

The gamma and αγ isoforms are classified as neuron-specific enolase (NSE). NSE is predominantly found in neurons and neuroendocrine cells and is a marker for tumors derived from these cells. It is used to monitor patients with small cell lung cancer. More recently, it has been used to monitor brain lesions. Monoclonal antibodies against γ-enolase have been produced in mice and used in the Cobas Core immunoassay system, which is a rapid, reliable and convenient test for measuring NSE levels in human serum [72].

New clinical requirements for triaging patients with chest pain are challenging the capabilities of existing cardiac markers. Serial mass measurements of creatine kinase isoforms, troponin forms, and myoglobin in emergency departments help to rapidly rule out acute myocardial infarction (AMI). However, during the first 3–4 hours after the onset of chest pain, their sensitivity is not high enough to make a significant contribution to the diagnosis of AMI. Proposed molecular markers for the early diagnosis of AMI include the αβ isoform of enolase [73].

Recommendations for Clinical Use of Enolase

The American Academy of Neurology has recommended the use of serum NSE to predict adverse outcome after global cerebral hypoperfusion in patients requiring cardiopulmonary resuscitation. However, limited availability has delayed the general use of this test for clinical decision making after global cerebral hypoperfusion [74].

A study was conducted to determine NSE levels relevant to neurological prognosis at 24, 48, and 72 hours after return of spontaneous circulation (ROSC) in a cohort of out-of-hospital cardiac arrest patients to validate previously proposed cut-off values, including ERC guidelines 2021. The results of studies using serum NSE levels to predict long-term adverse neurological outcome after out-of-hospital cardiac arrest showed higher NSE thresholds than suggested by previous publications [75].

A prospective study has also been conducted to investigate the prognostic efficacy of automated quantitative pupillometry in unconscious patients resuscitated from cardiac arrest. The validation of pupillometry combined with an NCE criterion of >60 μg/L is expected to increase the level of evidence for clinical prognosis [76].

There are no convincing data on the use of NSE in other areas of practical medicine (oncology, pediatrics).

There is no evidence to support the use of serum NSE for the diagnosis and monitoring of neuroblastoma. There is a high risk of false-positive results due to associated factors (e. g., hemolysis of the sample) and other conditions (e. g., inflammation), which significantly reduces the diagnostic value of this test [77].

Biomarkers cannot be used in standard pediatric monitoring due to a number of limitations. The main limitations are the heterogeneity of neurological complications, small cohort sizes, lack of multicenter studies, use of different neurobiomarker

evaluation methods, lack of consensus on biofluid assay validation, and lack of reference scores based on specific marker measurement techniques in biofluids [78].

Nevertheless, research into the potential applications of enolases in clinical practice continues. Mapping of specific epitopes yielded 32 most likely epitopes for enolase [79].

Several autism-specific NSE epitopes have been found in both mothers and newborns that can be used as biomarkers for the disease [80].

Methodological Flaws in Enolase Research

Despite years of research on brain injury markers, their use for stroke diagnosis, monitoring, and outcome prediction has not been translated into clinical practice [81]. Initially, these studies were based on biochemical techniques (isolation of molecular forms on columns, electrophoresis, etc.).

The enzyme-linked immunosorbent assay (ELISA) method was introduced in the 1960s and gained popularity in the 1970s and 1980s. This led to the gradual replacement of traditional biochemical research methods with ELISA, which we believe was a serious methodological error. ELISA is a very sensitive method. However, its high susceptibility to interference often results in errors that lead to erroneous conclusions and subsequent incorrect practical decisions [82].

Conventional immunoassays do not distinguish between isoenzymes [83, 84]. Analyzing the individual molecular forms of enolase has been largely abandoned. The clinical applicability of NSE quantification using conventional sandwich immunoassays is limited by lack of inter-assay agreement and falsely elevated concentrations due to hemolysis [85].

One error is the measurement of the total amount of homo- and heterodimers of the γ- and γα-isoforms of enolase.

Grouping γ-enolase and αγ-enolase as «neuron-specific enolase» is a flawed approach because they have different structures, are produced by different cells, and may have different functional properties. In this regard, the results of different ELISA-based studies of NSE in acute cerebrovascular accidents are highly inconsistent.

αγ enolase may combine the properties of α and γ subunits, but may also have properties that distinguish it from homodimeric forms of γ and α enolase. As a result, analytical methods involving sample preparation by immunoextraction of all molecular forms of NSE are being developed to study the different molecular forms of enolase [86, 84].

The primary difference between α-enolase and γ-enolase is their sensitivity to chloride ions, urea and temperature. Alpha-enolase is highly sensitive to chloride ions, urea, and temperature. In contrast, γ-enolase is more resistant to chloride-induced inactivation. Chloride ions accumulate in neurons during repetitive depolarization, making the relative insensitivity of γ-enolase to them an intriguing finding. The resistance of γ-enolase to chloride ions may have evolved to adapt to the intracellular environment of neurons, preventing inactivation of chloride-sensitive enolase when metabolic energy is most needed [84, 15].

It has been suggested to study the αγ heterodimer using antibodies against one subunit as a solid phase antibody and antibodies against the other subunit as a labeled complex [87].

Standardization of research methods is critical, including the use of reagents from the same company, the use of the same type of equipment to evaluate study results, and adherence to the chosen variant of biological sample study (sample preparation, elimination of cross-reactions, etc.).

The failure to measure the enzymatic activity of enolase was a significant methodological error. The point is that enolase is an enzymatic protein of glycolysis that produces energy under hypoxia and has cytoprotective properties.

Conclusion

The study of various molecular forms of enolases is an important trend in critical care medicine. This is because glycolysis plays a key role in the metabolism of various organs and systems during critical illness. When planning scientific research in this area, the following factors seem to be important.

First, the enzymatic activity of enolases in general and of each molecular form under investigation must be determined,

Second, antibodies against molecular forms of enolases label a specific subunit of the protein (α, γ or β). For example, antibodies to the γ subunit will detect both γ enolase and αγ enolase. Obviously, detection of both molecular forms of enolase does not allow assessment of their true levels and enzymatic activity. The solution to this problem is to isolate the enolases into single molecular forms that can be studied separately.

The notion that heterodimers are cell-specific isoenzymes is not supported by studies that have found them in cells from different organs. This certainly prevents personalized assessment of changes in molecular forms of enolases in various diseases (including critical illness).

Studies on a novel platform using methods to assess the level and enzymatic activity of each molecular form of enolase will shed light on the role of specific isoenzymes in disease pathogenesis, as well as the diagnostic and prognostic value of therapeutic

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